REMARKS

Claims 1-39 are pending. By this Amendment, claim 34 of non-elected Group III is amended to depend from claim 1. No new matter is added.

Applicants thank the Examiner for the indication that the claims of Group III (claims 34-37) will be rejoined at the time the product claims of Group I are found allowable. In order to expedite rejoinder, Applicants have amended claim 34 to read "A method of synthesizing the compound of claim 1, said compound being of formula (I):.." Also, Applicants respectfully submit that claims 2 and 7 should be rejoined with the claims of Group I, which are allowable for the reasons discussed below.

The Office Action rejects claims 1, 3-6 and 8-14 under 35 U.S.C. § 103(a) as being unpatentable over Wester et al. (J Nucl. Med., 1999) in view of Coenen et al. (U.S. Patent No. 4,925,651) and Tomiyoshi (Nucl. Med. Conn. 1997). These references and a similar rejection were discussed in Applicants' June 3, 2003, Amendment. This rejection is traversed.

The Office Action asserts that "[t]he compound disclosed by Wester et al. is the same as that claimed ...except for the methyl group, i.e., Wester discloses radiolabled tyrosine and radiolabeled methyl tyrosine is claimed" (see the third sentence in the second paragraph at page 3 of the Office Action). The Office Action further asserts that "Coenen and Tomiyoshi are relied upon for teaching [that] methyl tyrosine is equivalent to tyrosine..." (see the third sentence in the fourth paragraph on page 3 of the Office Action). According to the Office Action, "[s]uch substitution is known in the art to be a

structurally obvious modification to gain the advantage of obtaining analogous chemically related compounds" (see the fifth sentence in the fourth paragraph on page 3 of the Office Action).

However, Applicants cannot locate any specific teaching or suggestion in either of Coenen et al. or Tomiyoshi et al. that methyl tyrosine is equivalent to tyrosine.

However, Applicants do respectfully note that in the September 23, 2002, Office Action, the Examiner asserted that the present application "contains claims directed to the following patentably distinct species of the claimed invention: the various species as encompassed by the claimed formula" (see the third paragraph on page 3 of the September 23, 2003, Office Action). Thus, it would appear from the Examiner's assertion that a methyl tyrosine species of the present invention would be patentably distinct from a compound not containing a methyl tyrosine.

Thus, Applicants submit that the present claims are directed to a patentably distinct compound and should not have been rejected. Applicants further respectfully submit that this is particularly true for the "patentably distinct" species of claim 14.

Furthermore, as mentioned above, Coenen et al. nowhere teaches or suggests the equivalency of tyrosine and methyl tyrosine. However, Coenen et al. actually do demonstrate that a compound having a methyl tyrosine is **patentable** over a known tyrosine compound without the "methyl".

In particular, Applicants respectfully note that, during the prosecution of the Coenen et al. patent, "2-[^{18}F]-fluoro-a-methyl-tyrosine" was determined by the U.S. Patent and Trademark Office to be separately patentable over the known "2-[^{18}F]fluorotyrosine" (see page 275, lines 2-4, of the attached reference Chirakal et al., "Synthesis of 2- and 3-Fluorotyrosine with Dilute Fluorine Gas," Journal of Fluorine Chemistry, vol. 37 (1987) pp. 267-278, which was cited during prosecution of Coenen et al.).

Applicants have also attached hereto a copy of the Notice of Allowance from the Coenen et al. patent application which, at the top of page 3, states that "the prior art of record does not anticipate or render obvious, either singly or when combined together, the claimed radiolabelled compound...The prior art fails to teach such and there is no motivation to modify the compounds of the prior art to recite the claimed compound."

Thus, contrary to the assertion in the Office Action, Coenen et al. actually demonstrates that a methyl tyrosine compound is patentably distinct from tyrosine compound.

For at least the above reasons, reconsideration and withdrawal of the rejection of claims 1, 3-6 and 8-14 under 35 U.S.C. § 103(a) are respectfully requested.

Applicants respectfully submit that this application is in condition for allowance and such action is earnestly solicited. If the Examiner believes that anything further is desirable in order to place this application in even better condition for allowance, the Examiner is invited to contact Applicants' undersigned representative at the telephone

number listed below to schedule a personal or telephone interview to discuss any remaining issues.

In the event this paper is not considered to be timely filed, Applicants respectfully petition for an appropriate extension of time. The Commissioner is authorized to charge payment for any additional fees which may be required with respect to this paper to Counsel's Deposit Account 01-2300, **referring to client-matter number 107380-00005**.

Respectfully submitted,

Robert K. Carpenter
Registration No. 34,794

Customer No. 004372
ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
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RKC/tdd

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PART B - ISSUE FEE TRANSMITTAL

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE. Blocks 2 through 6 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advanced orders and notification of maintenance fees will be mailed to addressee entered in Block 1 unless you direct otherwise, by: (a) specifying a new correspondence address in Block 3 below; or (b) providing the PTO with a separate "FEE ADDRESS" for maintenance fee notifications with the payment of Issue Fee or thereafter. **See reverse for Certificate of Mailing.** 16881-104

MAR 1990 PATENT & TRADEMARK OFF.

1. CORRESPONDENCE ADDRESS	2. INVENTOR(S) ADDRESS CHANGE (Complete only if there is a change)
FOLEY & LARDNER SCHWARTZ, JEFFERY, SCHWAAB, MACK, BLUMENTHAL & EVANS P.O. BOX 299 ALEXANDRIA, VA 22313-0299	INVENTOR'S NAME Street Address City, State and ZIP Code CO-INVENTOR'S NAME Street Address City, State and ZIP Code ☐ Check if additional changes are on reverse side

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
07/280,804	12/07/88	005	MAPLES, J 223	12/19/89

First Named Applicant: COENEN, HEINZ H.

TITLE OF INVENTION: RADIOFLUORO-TYROSINE DERIVATIVES, THE PREPARATION AND USE THEREOF (AS AMENDED)

	ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
1	88COE1194RLS	424-001.001	561	UTILITY	NO	$620.00	03/19/90

3. Further correspondence to be mailed to the following:
FOLEY & LARDNER, SCHWARTZ, JEFFERY, SCHWAAB
MACK, BLUMENTHAL & EVANS
Suite 510, 1800 Diagonal Road
P.O. Box 299
Alexandria, Virginia 22313-0299

4. For printing on the patent front page, list the names of not more than 3 registered patent attorneys or agents OR alternatively, the name of a firm having as a member a registered attorney or agent. If no name is listed, no name will be printed.
1 FOLEY & LARDNER,
2 SCHWARTZ, JEFFERY, SCHWAAB, MACK,
3 BLUMENTHAL & EVANS

DO NOT USE THIS SPACE

120 03/09/90 07280804 1 142 620.00 CK

5. ASSIGNMENT DATA TO BE PRINTED ON THE PATENT (print or type)
(1) NAME OF ASSIGNEE: Kernforschungsanlage Juelich
03 Gesellschaft mit beschrankter Haftung
(2) ADDRESS: (City & State or Country) (D-5170) Juelich,
DEX Federal Republic of Germany
(3) STATE OF INCORPORATION, IF ASSIGNEE IS A CORPORATION

A. ☐ This application is NOT assigned.
XX☒ Assignment previously submitted to the Patent and Trademark Office.
☐ Assignment is being submitted under separate cover. Assignments should be directed to Box ASSIGNMENTS.

PLEASE NOTE: Unless an assignee is identified in Block 5, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

6a. The following fees are enclosed:
☒ Issue Fee ☐ Advanced Order - # of Copies _____ (Minimum of 10)

6b. The following fees should be charged to:
DEPOSIT ACCOUNT NUMBER _____
(Enclose Part C)
☐ Issue Fee ☐ Advanced Order - # of Copies _____ (Minimum of 10)
☐ Any Deficiencies in Enclosed Fees

The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee to the application identified above.

(Signature of party in interest of record) Richard L. Schwaab; Reg. No. 25,479
Barbara A McDowell Reg #31,640
(Date) March 7, 1990

NOTE: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

TRANSMIT THIS FORM WITH FEE-CERTIFICATE OF MAILING ON REVERSE

PTOL-85B (REV 12-88)(OMB Clearance is pending)

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: Box ISSUE FEE
COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

FOLEY & LARDNER
SCHWARTZ, JEFFERY, SCHWAAB, MACK,
BLUMENTHAL & EVANS
P.O. BOX 299
ALEXANDRIA, VA 22313-0299

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

☐ Note attached communication from the Examiner
☐ This notice is issued in view of applicant's communication filed _____

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
07/280,804	12/07/88	005	MAPLES, J 223	12/19/89
First Named Applicant	COENEN,	HEINZ H.		

TITLE OF INVENTION: RADIOFLUORO-TYROSINE DERIVATIVES, THE PREPARATION AND USE THEREOF (AS AMENDED)

	ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
1	88COE1194RLS	424-001.001	B61	UTILITY	NO	$620.00	03/19/90

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.

HOW TO RESPOND TO THIS NOTICE:

I. Review the SMALL ENTITY Status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:
A. If the Status is changed, pay twice the amount of the FEE DUE shown above and notify the Patent and Trademark Office of the change in status, or
B. If the Status is the same, pay the FEE DUE shown above.

If the SMALL ENTITY is shown as NO:
A. Pay FEE DUE shown above, or
B. File verified statement of Small Entity Status before, or with, payment of 1/2 the FEE DUE shown above.

II. Part B of this notice should be completed and returned to the Patent and Trademark Office (PTO) with your ISSUE FEE. Even if the ISSUE FEE has already been paid by a charge to deposit account, Part B should be completed and returned. If you are charging the ISSUE FEE to your deposit account, Part C of this notice should also be completed and returned.

All communications regarding this application must give series code (or filing date), serial number and batch number. Please direct all communications prior to issuance to Box ISSUE FEE unless advised to the contrary.

REMINDER: Patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees.

(JMB Clearance is pending)

PATENT AND TRADEMARK OFFICE COPY

Copy 12-15-89

UNITED S[illegible]EPARTMENT OF COMMERCE
Patent and [illegible]ark [illegible]ffice
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATT RNEY DOCKET NO.

EXAMINER	
ART UNIT	PAPER NUMBER
	8

DATE MAILED:

NOTICE OF ALLOWABILITY

PART I.

1. ☒ This communication is responsive to THE 5/24/89 AND 6/27/89 COMMUNICATIONS.
2. ☒ All the claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice Of Allowance And Issue Fee Due or other appropriate communication will be sent in due course.
3. ☒ The allowed claims are 5, 13-16.
4. ☐ The drawings filed on _____ are acceptable.
5. ☒ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has-[☑ been received. [_] not been received. [_] been filed in parent application Serial No. _____, filed on _____.
6. ☒ Note the attached Examiner's Amendment.
7. ☐ Note the attached Examiner Interview Summary Record, PTOL-413.
8. ☒ Note the attached Examiner's Statement of Reasons for Allowance.
9. ☒ Note the attached NOTICE OF REFERENCES CITED, PTO-892.
10. ☒ Note the attached INFORMATION DISCLOSURE CITATION, PTO-1449.

PART II.

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" indicated on this form. Failure to timely comply will result in the ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

1. ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.
2. ☐ APPLICANT MUST MAKE THE DRAWING CHANGES INDICATED BELOW IN THE MANNER SET FORTH ON THE REVERSE SIDE OF THIS PAPER.
   a. ☐ Drawing informalities are indicated on the NOTICE RE PATENT DRAWINGS, PTO-948, attached hereto or to Paper No. _____. CORRECTION IS REQUIRED.
   b. ☐ The proposed drawing correction filed on _____ has been approved by the examiner. CORRECTION IS REQUIRED.
   c. ☐ Approved drawing corrections are described by the examiner in the attached EXAMINER'S AMENDMENT. CORRECTION IS REQUIRED.
   d. ☐ Formal drawings are now REQUIRED.

Any response to this letter should include in the upper right hand corner, the following information from the NOTICE OF ALLOWANCE AND ISSUE FEE DUE: ISSUE BATCH NUMBER, DATE OF THE NOTICE OF ALLOWANCE, AND SERIAL NUMBER.

Attachments:

- ☑ Examiner's Amendment
- _ Examiner Interview Summary Record, PTOL-413
- ☑ Reasons for Allowance
- ☑ Notice of References Cited, PTO-892
- ☑ Information Disclosure Citation, PTO-1449
- _ Notice of Informal Application, PTO-152
- _ Notice re Patent Drawings, PTO-948
- _ Listing of Bonded Draftsmen
- _ Other

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 5 and 13-16, drawn to a radioactive tyrosine compound and method of use, classified in Class 424, subclass 1.1.

II. Claims 6-12, drawn to a method of making a radioactive tyrosine compound, classified in Class 562, subclass 445.

2. The inventions are distinct, each from the other because of the following reasons:

the compound of Group I could be made by a materially different process such as by first forming the claimed non-radioactive compound and then irradiating the fluorine to produce the claimed compound.

3. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification and recognized divergent subject matter, restriction for examination purposes as indicated is proper.

4. During a telephone conversation with Carol Williams on November 30, 1989 a provisional election was made without traverse to prosecute the invention of Group I, claims 5 and 13-16.

5. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Huffman et al. teach a protein containing a tyrosine moiety while Pawelek sets forth a radiolabeled phenylalanine compound.

6. The following is an Examiner's Statement of Reasons for Allowance:

December 14, 1989/jsm

the prior art of record does not anticipate or render obvious, either singly or when combined together, the claimed radiolabelled compound or the method of use of said compound. The prior art fails to teach such and there is no motivation to modify the compounds of the prior art to recite the claimed compound.

Any comments considered necessary by applicant must be submitted no later than the payment of the Issue Fee and, to avoid processing delays, should preferably accompany the Issue Fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

7. This application is in condition for allowance except for the presence of claims 6-12 to an invention nonelected without traverse. Accordingly, claims 6-12 have been cancelled.

8. An inquiry concerning this communication should be directed to John S. Maples at telephone number 703-557-1868.

John S. Maples

JOHN S. MAPLES
EXAMINER
ART UNIT 223

December 14, 1989/jsm

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Heinz Hubert COENEN et al

Serial No.: 07/280,804

Filed: December 7, 1988

For: RADIOHALOGENOTYROSINE DERIVATIVES, THE PREPARATION AND USE THEREOF

PRELIMINARY AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE CLAIMS:

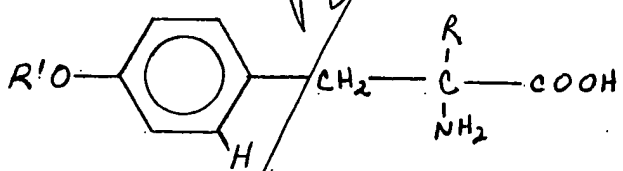
Claims 1-4: cancel the claims.

Please add the following claims:

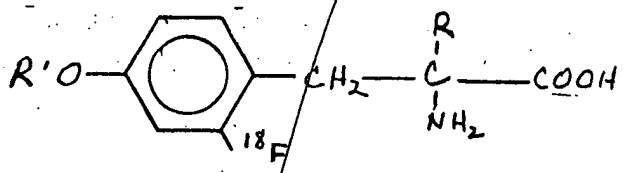
-- 5. Substantially pure 2-[^{18}F]-fluoro-a-methyltyrosine.

-- 6. A process for preparing substantially pure 2-[^{18}F]-fluorotyrosine or 2-[^{18}F]-fluoro-a-methyltyrosine, which comprises the steps of:

(a) reacting a compound having the formula:



wherein R represents hydrogen or methyl, and R' represents an acyl group, with an electrophilic radioactive fluorine species, to form an intermediate having the formula



wherein R is hydrogen or methyl and R' is an acyl group; and

(b) hydrolytically eliminating the acyl group from said intermediate to form said compound, and recovering said compound in substantially pure form. --

-- 7. The process of claim 6, wherein R' is an acetyl group. --

-- 8. The process of claim 6, wherein in step (b), said hydrolysis is effected in the presence of an alkali metal hydroxide. --

-- 9. The process of claim 8, wherein said alkali metal hydroxide is sodium hydroxide. --

-- 10. The process of claim 6, wherein in step (b), the product is purified by liquid chromatography. --

-- 11. The process of claim 6, wherein in step (a), sadi electrophilic radioactive fluorine species is $[^{18}F]$-F_2. --

-- 12. The process of claim 11, wherein the reaction of step (a) is effected in the further presence of trifluoroacetic acid. --

2 ~~13~~. In a method of emission-tomographically measuring protein synthesis in vivo by positron emission tomography or single photon emission tomography, the improvement comprising administering an amount, effective as a radio- tracer, of 2-[^{18}F]-fluorotyrosine or 2-[^{18}F]-fluoro-a-methyltyrosine.

3 ~~14~~. The method of claim 2 ~~13~~, wherein said compound is 2-[^{18}F]-fluorotyrosine.

4 ~~15~~. The method of claim 2 ~~13~~, wherein said compound is 2-[^{18}F]-fluoro-^{a}-methyltyrosine.

5 ~~16~~. The method of claim 2 ~~13~~, wherein said protein synthesis is measured in the brain.

end

REMARKS

The subject application is amended in light of references cited in the accompanying Information Disclosure Statement. Original claims 1-5 are cancelled, and new claims 6-16 are added by the present amendment. No new matter is believed to be added and entry of the amendments is respectfully requested.

An early action on the merits is earnestly solicited.

Respectfully submitted,

Date: May 24, 1989

Bernhard D. Saxe
Reg. No. 28,665

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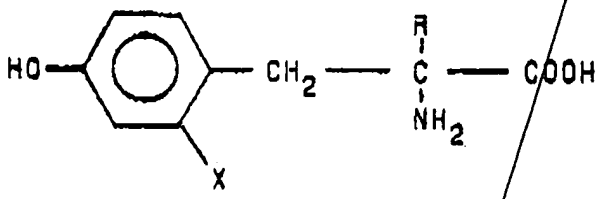
SCHWARTZ, JEFFERY, SCHWAAB,
MACK, BLUMENTHAL & EVANS
P.O. Box 299
Alexandria, Virginia 22313-0299
Tel: (703) 836-9300
Fax: (703) 683-4109

Patent claims

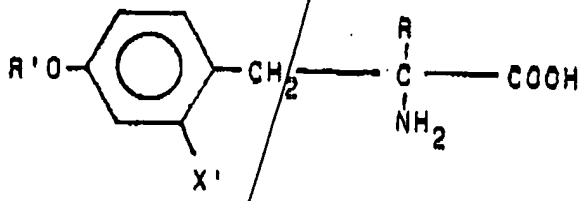
1. 2-Radiohalogenotyrosin derivatives of the formula I



(I)

in which X denotes a radioactive halogen, in particular fluorine-18, bromine-75 or iodine-123, and R denotes hydrogen or methyl.

2. A process for the preparation of the compounds as claimed in claim 1, which comprises a compound of the formula II



(II)

in which R has the meaning already indicated, and R′ is an acyl group, and X′ is hydrogen, halogen or an organometallic radical, being subjected either to electrophilic substitution with an electrophilic radioactive species of the halog n which is to be introduced in the

2-position - where appropriate with the addition of an oxidizing agent when radiobromide or radioiodide is used - when X' is hydrogen or an organometallic radical, or, when X' denotes halogen, to an exchange reaction with the desired radiohalide, and, after hydrolytic elimination of the acyl group, the compound of the formula I which has formed being isolated by chromatography.

3. The use of a compound as claimed in claim 1 as a tracer for emission-tomographic measurement of protein synthesis in vivo by means of positron emission tomography (PET) or single photon emission tomography (SPECT).

4. Use of 2-fluoro(18)-tyrosine and -methyltyrosine for PET investigations of protein synthesis in vivo, especially for the purposes of cerebral diagnosis.

Journal of Fluorine Chemistry, 37 (1987) 267–278

Received: May 1, 1987; accepted: June 29, 1987

SYNTHESIS OF 2- AND 3-FLUOROTYROSINE WITH DILUTE FLUORINE GAS

R. CHIRAKAL*, K.L. BROWN, G. FIRNAU, E.S. GARNETT

Nuclear Medicine, McMaster University Medical Centre

D.W. HUGHES, B.G. SAYER

Department of Chemistry

and R.W. SMITH

McMaster Regional Centre for Mass Spectrometry
McMaster University, 1200 Main St. W.,
Hamilton, Ont., L8N 3Z5 (Canada)

SUMMARY

Differences in reactivity and selectivity of fluorine gas towards L-tyrosine and the O,N-diacetylated derivative of L-tyrosine methyl ester have been exploited for the synthesis of 2- and 3-fluorotyrosine. Both 2- and 3-fluorotyrosine were identified by ^{1}H, ^{19}F and ^{13}C NMR spectroscopy and high resolution mass spectrometry. The short synthesis time and high reaction yields allow this procedure to be used for the incorporation of the short lived positron emitting radionuclide ^{18}F into the aromatic ring of L-tyrosine.

*Author to whom correspondence should be addressed.

0022-1139/87/$3.50

INTRODUCTION

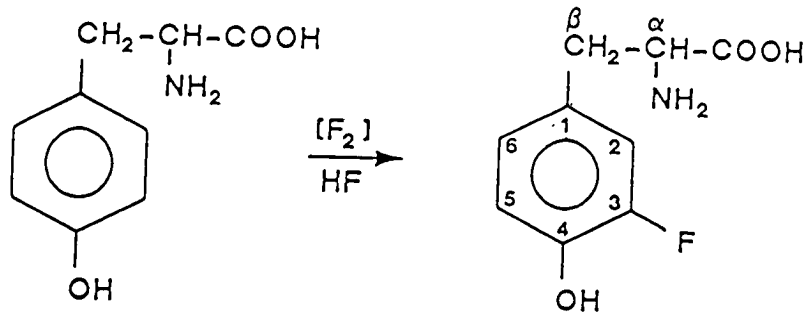
Tyrosine is a nonessential aromatic amino acid derived in animal cells by the hydroxylation of phenylalanine. It is a precursor of thyroxine and of the neurotransmitter hormones adrenaline and noradrenaline. It is also a precursor of the central neurotransmitter dopamine. In addition, it is a constituent of many proteins. If a suitable gamma emitting label can be found for tyrosine, it should be possible to use standard techniques of nuclear medicine to study hormone, neurotransmitter or protein synthesis. Carbon-11 ($T_{1/2}$ = 20 min) and nitrogen-13 ($T_{1/2}$ = 10 min) would be obvious labels but their relatively short half life would preclude their use in studies lasting beyond a couple of hours.

Fluorine-18 ($T_{1/2}$ = 110 min) has been used successfully to label 3, 4-dihydroxyphenylalanine [1] and the resulting 6-[^{18}F]fluorodopa has been used to visualize and quantitate the cerebral metabolism of dopamine in living human brain [2]. To date, ^{18}F has been introduced into the aromatic ring of tyrosine by the Schiemann reaction [3], a time-consuming multistep radiochemical synthesis that yields very small amounts (1-3%) of 3-fluorotyrosine. We now describe a method for a high yield synthesis of 3-fluorotyrosine by direct fluorination of tyrosine. We also report a method, using dilute fluorine gas, for the synthesis of 2- and 3-fluorotyrosine. The synthesis and identification of 2-fluorotyrosine has not been reported in the literature. The synthesis of 2-fluorotyrosine by direct fluorination requires modification of the reactivity and orientation of tyrosine towards electrophilic substitution. This has been achieved by the derivatization of the phenolic group. Because our methods produce high yields and short synthesis times, the procedures can also be applied for the incorporation of ^{18}F into tyrosine.

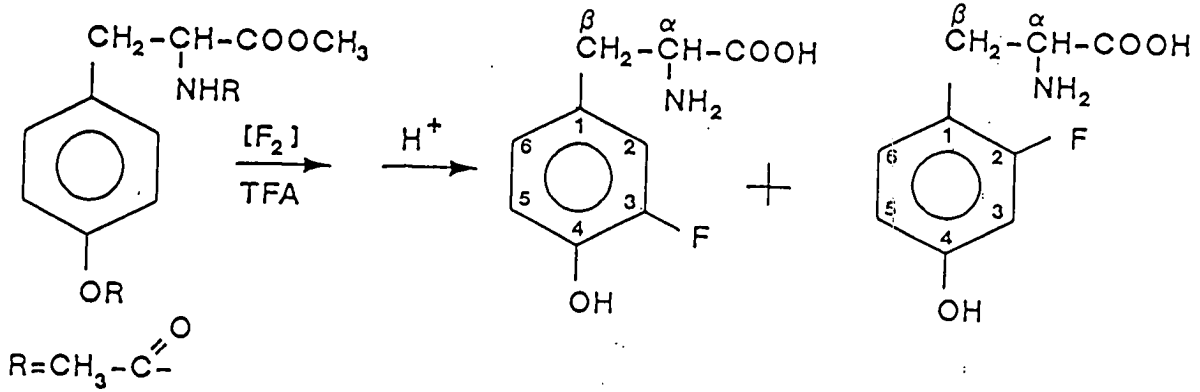
RESULTS AND DISCUSSION

Identification of products: HPLC analysis of the reaction mixture (from Scheme I) gave two UV peaks, a major one at 25 min and a second one at 33 min. A comparison of the retention time as well as spiking the sample with authentic tyrosine enabled us to assign the earlier peak to tyrosine. The sample eluting at 33 min had the same retention time and co-chromatographed with authentic 3-fluorotyrosine. The 33 min sample and 3-fluorotyrosine showed similar U.V. characteristics (λ max = 274 nm) and their ^{19}F NMR spectra consisted of multiplets at -136.6 ppm. (Fig. 1). ^{1}H NMR spectrum of the sample revealed one less proton in the aromatic region when compared with the spectrum of L-tyrosine. The ^{1}H chemical shifts and coupling constants were also similar to those of authentic 3-fluorotyrosine (Table 1). Carbon-13 chemical shifts of both compounds agreed with the values predicted from fluorine substituent shifts [4] (Table 2). In addition, the low and high resolution fast atom bombardment mass spectra of the sample were identical to those of 3-fluorotyrosine and showed protonated molecular ion at M/z = 200 $[(m+H)^{+}]$. The chemical yield, using F-18 labelled fluorine gas, was 46% with respect to ^{18}F-fluorine.

2-Fluorotyrosine was isolated along with 3-fluorotyrosine from reaction Scheme II. HPLC analysis of the reaction mixture showed major peaks for tyrosine, 3-fluorotyrosine and another peak at 34 min. We have ascribed the latter peak to 2-fluorotyrosine on the basis of the following observations. The high resolution mass spectrum of the combined peaks was identical to that of 3-fluorotyrosine. A radiochromatogram of the reaction mixture showed the ratio of F-18 activity in the peaks eluting at 33 and 34 min to be 62:38. A ^{19}F NMR spectrum of the combined peaks gave multiplets at -136.6 ppm (3-fluorotyrosine) and at -115.8 ppm (Fig. 1). The distribution of the



SCHEME I



SCHEME II

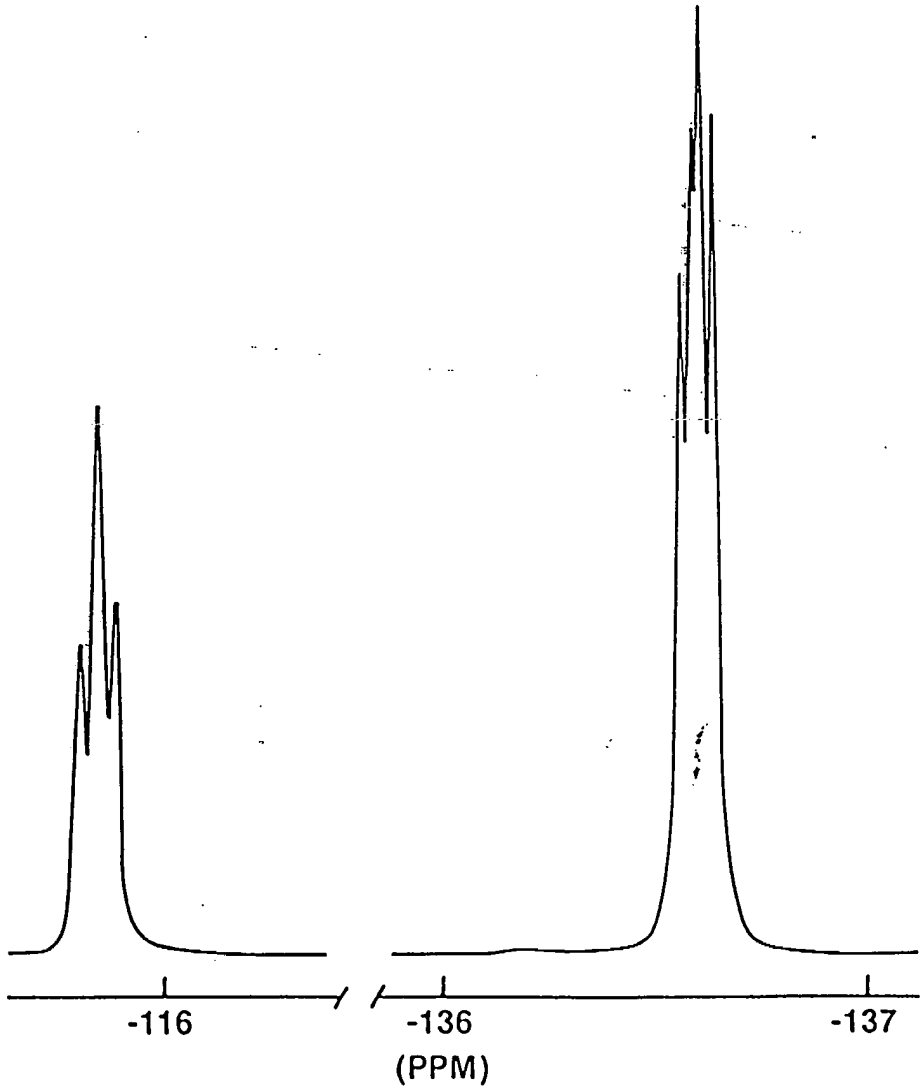


Fig. 1. ^{19}F spectrum of 3-fluorotyrosine (right) and 2-fluorotyrosine (left)

areas under these multiplets (60:40) agreed with the distribution of F-18 on the radiochromatogram. A carbon-13 spectrum of the mixture gave signals well resolved from those arising from 3-fluorotyrosine. Their

SCHEME II

$R=CH_3-\overset{\overset{O}{\|}}{C}-$

TABLE 1

^1H chemical shifts and coupling constants of 3-fluorotyrosine.

	Chemical Shifts (δ) ppm		Coupling Constants (Hz)	
	3-F tyrosine	33 min sample		
H-α	4.10	4.03	$^3J_{\alpha\beta}$	5.7
H-β	3.05	2.97	$^3J_{\alpha\beta'}$	7.5
H-β'	2.94	2.87	$^2J_{\beta\beta'}$	-14.8
H-2	6.86	6.79	$^4J_{H_2H_6}$	1.9
H-5	6.77	6.68	$^3J_{H_5H_6}$	8.2
H-6	6.75	6.70	$^3J_{H_2F}$	12.0
			$^3J_{H_5F}$	8.2

chemical shifts agreed with the values predicted for 2-fluorotyrosine (Table 2). The combined chemical yield of 3- and 2-fluorotyrosine using fluorine-18 fluorine gas was 26%.

Synthesis of 3-fluorotyrosine using the pyrolysis followed by acid hydrolysis of 2-methoxy-5 (2',2'-dicarbethoxy-2'-acetamidoethyl)-phenyl diazonium tetrafluoroborate [3] gives 3-fluoro-DL-tyrosine which is of limited use for biological and pharmaceutical investigations because the L-enantiomer is needed for these studies. It has been shown that electrophilic fluorination of the aromatic ring using F_2 [5], XeF_2 [6], and acetyl hypofluorite [7] does not affect the chiral centre on the side

TABLE 2

^{13}C chemical shifts (a) (ppm) and ^{13}C - ^{19}F coupling constants (b) (Hz) of 3- and 2-fluorotyrosine.

a) Chemical Shifts

	3-Fluorotyrosine		2-Fluorotyrosine
COOH	172.3	COOH	---
C_α	55.1	C_α	54.6
C_β	35.7	C_β	30.6
C_1	127.6 (127.6)*	C_1	113.5 (112.5)*
C_2	118.2 (117.8)	C_2	162.9 (167.1)
C_3	152.5 (152.3)	C_3	104.4 (102.9)
C_4	144.0 (142.1)	C_4	158.2 (157.2)
C_5	119.4 (118.0)	C_5	113.1 (112.7)
C_6	127.0 (127.6)	C_6	133.6 (132.9)

b) Coupling Constants

$^3J_{1,F}$	5.7	$^2J_{1,F}$	16.1
$^2J_{2,F}$	18.4	$^1J_{2,F}$	243.8
$^1J_{3,F}$	240.7	$^2J_{3,F}$	25.0
$^2J_{4,F}$	12.8	$^3J_{4,F}$	12.3
$^3J_{5,F}$	---	$^4J_{5,F}$	2.3
$^4J_{6,F}$	2.6	$^3J_{6,F}$	5.7

*Calculated values using fluorine substituent parameters are shown in brackets.

chain. We, therefore, believe that the fluorotyrosine obtained from both reaction schemes is the L-enantiomer.

We [1] have shown that direct fluorination of catecholamines in anhydrous hydrogen fluoride produces monofluorinated products in high yields. Our results show that the fluorination of tyrosine in anhydrous

hydrogen fluoride is highly regiospecific. Similar results were obtained when the reaction was carried out in trifluoroacetic acid instead of hydrogen fluoride, even though the yield of 3-fluorotyrosine was reduced to 23%. An electrophilic substitution similar to the ionic halogenation reactions of aromatic compounds can be offered as a possible reaction mechanism. Formation of 3-fluorotyrosine (Scheme I) is consistent with this mechanism because the ortho and para directing nature of the -OH group in tyrosine would yield predominantly 3-fluorotyrosine. Misaki [8] has reported similar results when phenol and p-cresol were used as the substrate for direct fluorination.

Protection of the phenolic group by a less activating group towards electrophilic substitution, such as the acetyl group, should favor the formation of 2-fluorotyrosine. Indeed,the direct fluorination of O,N-diacetyltyrosine methyl ester in trifluoroacetic acid solution (Scheme II) produced both 2- and 3-fluorotyrosine. The ratio of 2- to 3-fluorotyrosine was the same even when the reaction was carried out in solvents such as CH_3CN or $CHCl_3$:$CFCl_3$ (1:1). However, the yields of 2- and 3-fluorotyrosine, when these solvents were used, were very low (1-3%).

When HF, rather than TFA, was the solvent, 98% of the monofluorinated product was 3-fluorotyrosine. This may be due to hydrolysis of the acetyl groups by HF, after which the fluorination reaction becomes similar to Scheme I. Use of acetyl hypofluorite as the fluorinating agent did not improve the regiospecificity of the reaction depicted in Scheme II. We are unable to assess how much steric hindrance from the side chain contributes to the orientation effect. If the fluorination proceeds predominantly by a 'polar substitution mechanism' [9], then the protection of the phenolic group by a more electronegative substituent should be more effective for the formation of 2-fluorotyrosine.

Reaction Scheme I provides an efficient and rapid (120 min) method for the production of ^{18}F labelled 3-fluorotyrosine. Our results show that 70 mCi of ^{18}F [F_2] is sufficient to produce 5 - 6 mCi of 3-[^{18}F]fluorotyrosine. We have demonstrated that the reactions in Scheme II can be used to make 2-fluorotyrosine. Separation of 2- and 3-fluorotyrosine requires improved HPLC conditions. Our preliminary results indicate that millicurie quantities of 2-[^{18}F]fluorotyrosine can be obtained by careful fractionation of the eluate.

EXPERIMENTAL

L-Tyrosine (Fisher Scientific), m-fluoro-DL-tyrosine (Sigma), hydrogen fluoride (Matheson), and trifluoroacetic acid (BDH) were obtained commercially and used without further purification.

NMR Spectroscopy

The ^1H NMR spectra were recorded at 500.13 MHz on a Bruker AM-500 spectrometer. When D_2O:DCl was used as solvent, the chemical shifts were reported with respect to HDO (4.6 ppm). When $CDCl_3$ was used as solvent, the residual chloroform signal (7.24 ppm) relative to TMS was used as an internal reference. ^{19}F spectra were recorded at 235.36 MHz on a Bruker WM-250 spectrometer. Samples were dissolved in D_2O:DCl and the chemical shifts were reported in ppm relative to the external $CFCl_3$ (0 ppm). Carbon-13 spectra were recorded on a Bruker AM-500 spectrometer at 125.76 MHz over a 30.0 KHz sweep width in 32 K data points. The ^{13}C chemical shifts were reported relative to external TMS. The ^{13}C chemical shifts of the aromatic carbons in 2- and 3-fluorotyrosine were calculated using fluorine substituent shifts [4] in ortho and meta positions of L-tyrosine.

Mass Spectrometry

High resolution mass spectra were obtained with a double focusing VG ZAB-E mass spectrometer under positive ion fast atom bombardment conditions. Trifluoroacetic acid (20%) in glycerol was used as the matrix and xenon was the bombarding species (8 KeV). Accurate masses and elemental composition of the protonated molecular ions were determined under high resolution conditions (resolution 6000), using glycerol cluster ions as the reference.

HPLC analyses

HPLC analyses were done using two semi-preparative reverse phase columns (Waters μ-Bondapak C_{18}, 7.8 mm x 300 mm) with 0.1% acetic acid as mobile phase at 2 ml/min. The eluate was monitored for UV absorption at 280 nm. In experiments where fluorine-18 was used to determine the yield of the reaction with respect to fluorine, the eluate was also passed through a sodium iodide detector. For identification purposes, the eluates were co-chromatographed and their retention times were compared with those of authentic L-tyrosine and 3-fluoro-DL-tyrosine, (25 and 33 minutes respectively).

Preparation of L-methyl-N-acetyl-(4-acetoxyphenyl)alanine (1)

A stream of HCl gas was bubbled through a cold ($0^{o}C$) suspension of L-tyrosine (3.9 g) in methanol for 1-2 min. The resultant clear solution was stirred overnight. The solvent was evaporated, the residue was redissolved in methanol and evaporated again. The residue was then dried overnight to give L-tyrosine methyl ester hydrochloride (4.5 g, 88% yield). The crude tyrosine methyl ester HCl was stirred, with cooling, in a mixture of pyridine-acetic anhydride (1:1, 25 ml) for 10 min. The mixture was poured into 1 M sulphuric acid (100 ml) and the product was

extracted with ethyl acetate (3 x 60 ml) to yield a white solid (6.0 g). This was recrystallized from ethyl acetate, filtered and washed with hexane-ethyl acetate (1:2) to give 5.2 g of white solid, mp 98-100°C. The ^1H NMR spectrum of (1) in $CDCl_3$ comprised signals at 1.90 (S, 3H), 2.20 (S, 3H), 3.02 (2H, ABX Pattern J_{AB} = -13.70 Hz, J_{AX} = J_{BX} = 5.70 Hz), 3.65 (S, 3H), 4.79 (m, 1H J_{CH-NH} = 7.3 Hz), 6.13 (broad doublet 1H) and 6.93-7.03 (4H AA' BB'). A high resolution mass spectrum of the compound gave a major peak at M/z 280 $[m+H]^+$.

3-Fluorotyrosine: Reaction Scheme I

L-Tyrosine (100 mg) was placed in a Kel-F vessel and hydrogen fluoride was condensed into it under vacuum at -196°C. The mixture was degassed and equilibrated at -65°C. Dilute fluorine gas (0.5% in neon) was bubbled through the solution at approximately 70-90 ml/min for 30 minutes. Hydrogen fluoride was then removed by vacuum distillation. A brown residue was obtained. This was dissolved in 1 M HCl and transferred into a round bottomed flask. The solvent was evaporated on a rotary evaporator and the residue was washed with water and evaporated again. The final residue was dissolved in 2 ml of water and filtered through a 0.4 μ filter for HPLC separation.

2-Fluorotyrosine: Reaction Scheme II

Compound (1) (150 mg) was dissolved in 6-8 mL of trifluoroacetic acid. The solution was kept at 0-4°C and 0.5% fluorine in neon was bubbled through at 70-90 ml/min for 30 min. The reaction mixture was transferred into a round bottomed flask and the solvent was evaporated under vacuum. The yellowish oily residue that was obtained was dissolved in 48% HBr and refluxed for 25 minutes at 145°C. The HBr was evaporated, and the residue was washed with water (2 x 5 ml portions) and evaporated to remove any traces of acid. The final residue was dissolved in 2 ml of water and filtered through 0.4 μ filter for HPLC analysis.

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